SECTION II: KINETICS AND BIOREACTOR DESIGN:

LESSON 9.1. - Enzymatic kinetics, microbial kinetics and metabolic stoichiometry - Brief review on enzymatic reaction kinetics

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AIMS FOR TODAY'S LESSON

1.- ABOUT KINETICS (again, not kidding):

Reviewing chemical kinetics and terminology.

2.- ABOUT RATES:

reaction rates // production rates.

3.- ABOUT KINETIC MODELS:

What a model is.

Kinds of models.

REFERENCES:

- **Bailey, J.E., Ollis D.F.** (1986), *Biochemical Engineering Fundamentals*, McGraw-Hill (New York).

- **Doran, P.M.** (2013), *Bioprocess Engineering Principles*, Academic Press (London).

ISSUES IN THIS UNIT

WHAT WE ARE GOING TO TALK ABOUT…

KINETICS:

RATES:

KINETICS MODELS:

WHAT WE ARE GOING TO TALK ABOUT…

KINETICS:

- **Definition**
- **Aims**

RATES:

KINETICS MODELS:

WHAT WE ARE GOING TO TALK ABOUT…

KINETICS:

RATES:

Reaction rate

Production rate

Mass Balance and rates.

KINETIC MODELS:

WHAT WE ARE GOING TO TALK ABOUT…

KINETICS:

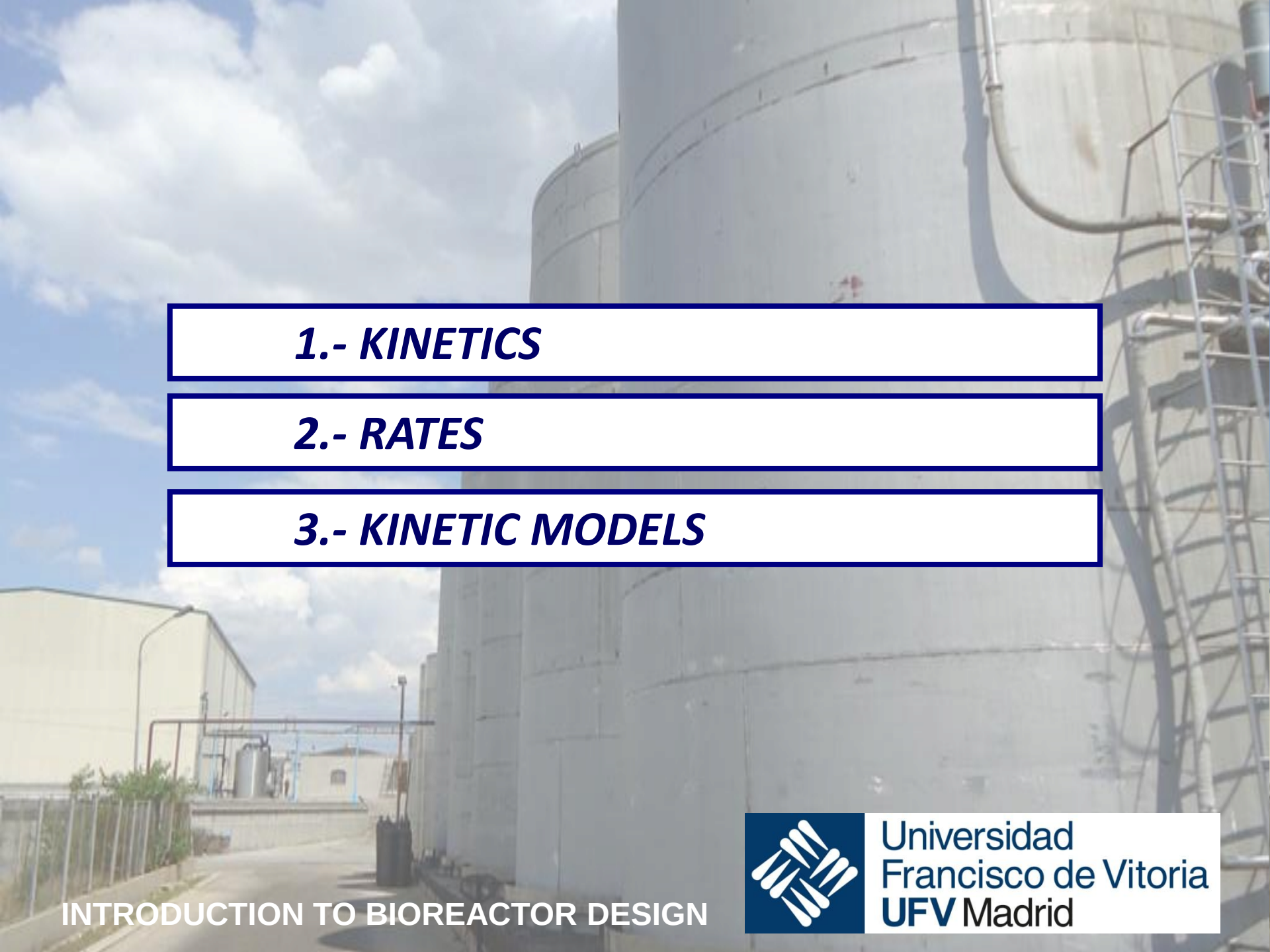
RATES:

KINETICS MODELS:

Definition of "model".

What for?

Kinds of models.



1.- KINETICS

2.- RATES

3.- KINETIC MODELS

1.- KINETICS

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1. KINETICS

- Etymologically, "**KINETICS**" ← "**Κινη**" "**Kiné**" :

movimiento

DEFINITION:

Part of chemistry concerned about the study of evolution of reactions, their rate and the different factors that can affect it.

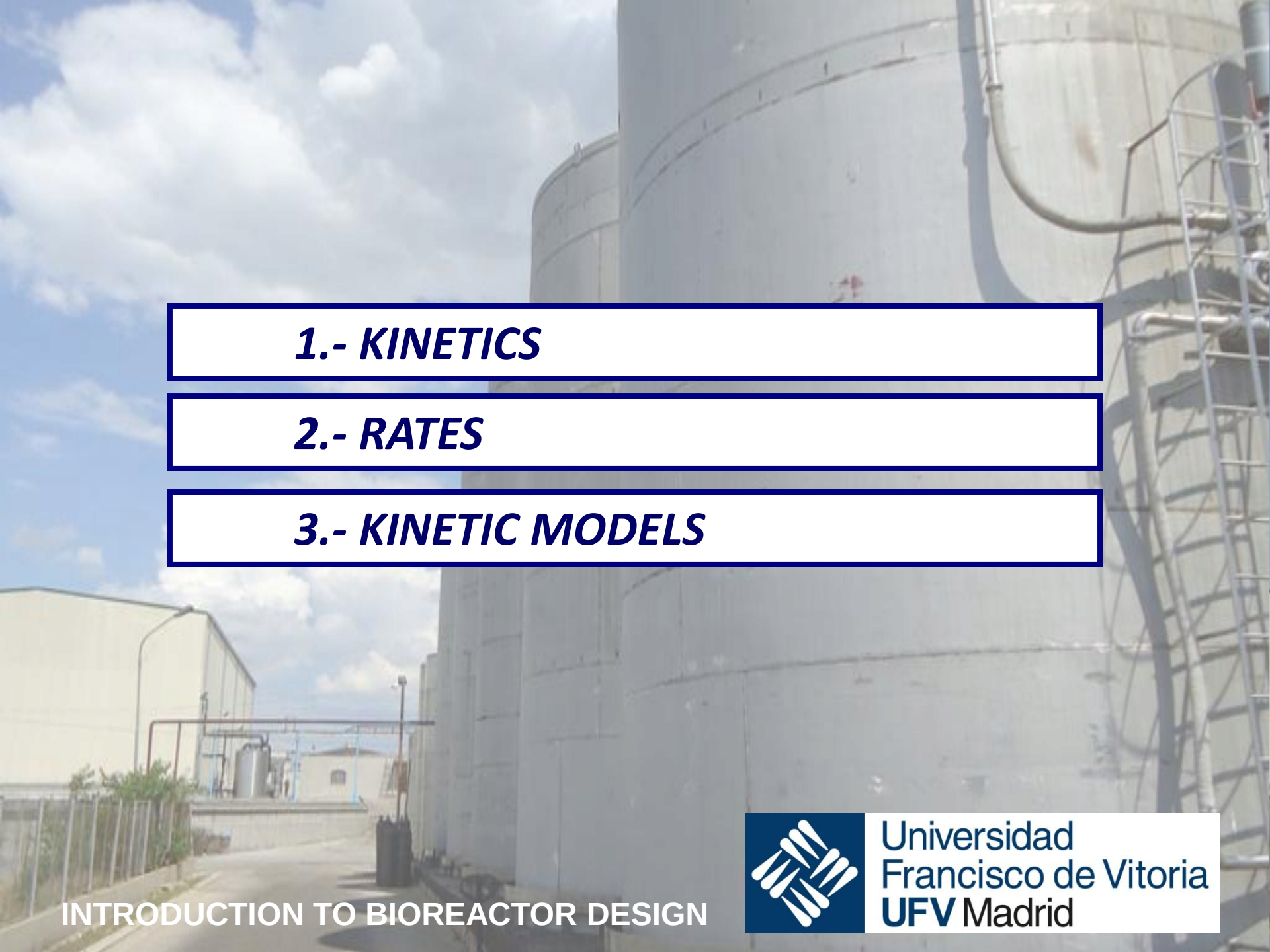
1. KINETIC AIMS

1. Establish the mechanism of a reaction
2. Know molecular structures
3. Study bond formation / breakage
4. Infer the relationship between reaction rate and process variables (temperature, concentration, pressure, etc.)

➔ **Chemical Reaction Engineering**

(Applied Chemistry Kinetics)

KINETIC MODEL



1.- KINETICS

2.- RATES

3.- KINETIC MODELS

2.- RATES

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2. RATES

Example:

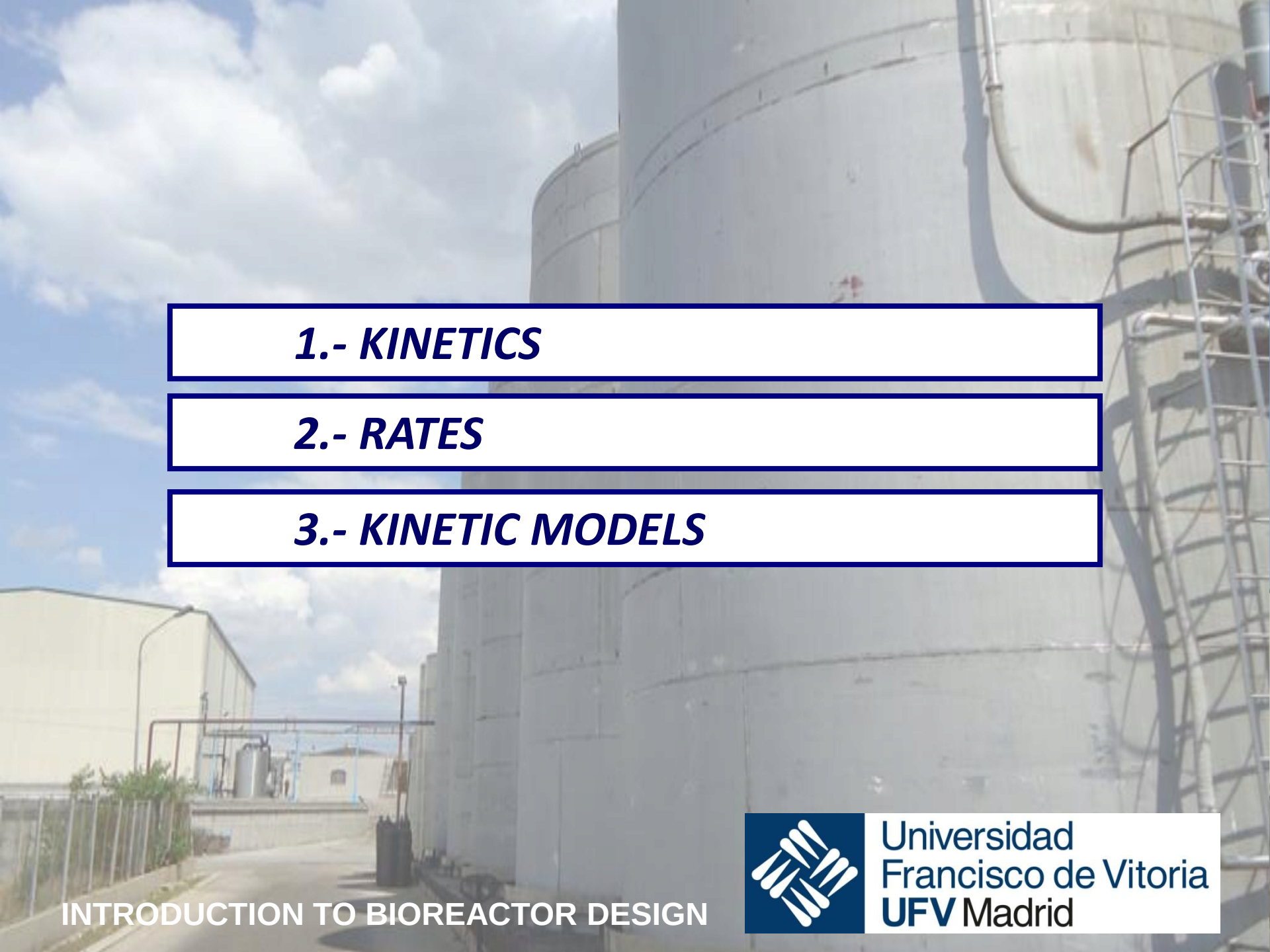
Reaction network

or metabolic path

$$aA + bB \rightarrow qQ + sS, \quad r_1$$
$$cC + b'B \rightarrow tT, \quad r_2$$
$$dD + s'S \rightarrow a'A + t'T, \quad r_3$$

$$R_j = \sum_{i=1}^{N} \nu_{j,i} \cdot r_i$$

$$R_A = -a \cdot r_1 + a' \cdot r_3$$
$$R_B = -b \cdot r_1 - b' \cdot r_2$$
$$R_C = -c \cdot r_2$$
$$R_D = -d \cdot r_3$$
$$R_S = s \cdot r_1 - s' \cdot r_3$$
$$R_Q = q \cdot r_1$$
$$R_T = t \cdot r_2 + t' \cdot r_3$$



1.- KINETICS

2.- RATES

3.- KINETIC MODELS

3.- KINETIC MODELS

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3. KINETIC MODELS

KINETIC EQUATION: algebraic expression able to predict, quantitative talking, the relatioship between **REACTION RATE** and **VARIABLES** affecting it.

In order to obtain this equation **KINETIC PARAMETERS** need to be established.

KINETIC MODEL: Set of kinetic equations for each reaction in a **REACTION NETWORK**.

3. KIND OF KINETIC MODELS

According the way the are obtained:

1. **Empirical models:** statistical relationship between variables, r=f(C,T) by data fitting.
2. **Mechanistic models:** deduced equations from an hypothetical mechanism.

According the kind of kinetic equation:

1. **Potential models:** variables can be separated, elemental reactions

$$r = f(Composition) = k \cdot [A]^{n1} \cdot [B]^{n2} \cdots$$

2. **Hyperbolic models:** variables cannot be separated. Non elemental reactions.

$$r = f(Composition) = \frac{k \cdot [A]^{n1} \cdot [B]^{n2} \cdots}{1 + K_A \cdot [A]^{n1} + K_B \cdot [B]^{n2} \cdots}$$

3. KINETIC MODELS

THEORY: Mechanism

EXPERIMENTS:

Obtaining experimental data

- INTERPRETATION OF EXPERIMENTAL DATA
  - ESTIMATION PARAMETERS
  - MODEL DISCRIMINATION
  - VALIDATION OF THE MODEL
- EQUIPMENT
- EXPERIMENTATION
- ANALYSIS

3. KINETIC MODEL DETERMINATION

Starting point:

Choosing the best approach in order to describe the evolution of our system:

a) **Theorical approach**: using predictive models. Broadly speaking results are less realistic.

b) **Empirical approach**: Chemical Reaction Engineering builds empirical models from experimental data.

3. KINETIC MODEL DETERMINATION

Starting point:

b) **Empirical approach**: steps ➔

1) Thinking up the experimental system:

Phase contact: study little by little: 1 phase, several phases

Identification of Rate-determining step, Stoichiometry, Thermodynamics.

2) Data Generation

Issues: experimental equipment (**batch, continuous, semicontinuous**), experimental conditions, experimental design and instrumental facilities.

3. KINETIC MODEL DETERMINATION

Starting point :

b) **Empirical approach**: steps ➔

3) Data interpretation and analysis

Issues:

- Proposing different candidate models
- Mathematic transformation of models
- Kinetic parameters calculation and statistics
- Model discrimination (choosing the most appropiate one)
- Validation of model: accuracy.

3. KINETIC MODEL DETERMINATION

- **Kinetic parameters calculation and statistics**.

  Classic calculus methods: differential / integral.

  Fitting methods: simple or multiple regression (linear or non linear)

- **Model discrimination**:

  Physical criteria

  Statistical criteria:

  Confidence level.

  Statistical significance of fitting.

- **Validation**: accurancy ← **Analysis of variance (ANOVA), residual analysis**

3. KINETIC MODELS

ENZYMATIC PROCESSES

One single reaction (non reversible)
Easy Reaction monitoring
Relatively stable Catalyst

$$S_1 + S_2 + S_3 + \ldots \xrightarrow{Enzyme} P_1 + P_2 + \ldots$$

KINETIC MODEL

Only one kinetic equation

Mechanistic models

Inhibition (Regulation)

Deactivation

Efects: T, pH

Agregation, proteolysis,…

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

$$r = \frac{\nu_{max} \cdot [S]}{K_M + [S]}$$

Mechanistic kinetic model
Only one reaction rate
Very simple reaction scheme

3. KINETIC MODELS

ALIVE CELLS IN BIOPROCESSES

STOICHIOMETRY

Many enzymatic reactions: Metabolism
Complex scheme of reactions: need simplification
ANALYSIS: stoichiometric study

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{CELLS}$$

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{Products}$$

$$\text{Substrates} \xrightarrow{\text{Cells}} \text{Energy}$$

KINETIC MODELS

Each **KEY COMPUND** for each reaction
Autocatalytic reactions
Slow process ➔ higher reactor volume or reaction time
Depending on cell type: chemo-, photo-, heterotroph, autotroph
O_2 (aerobic, anaerobic), T, pH
cell state: phase growth, viability, stability (GMO)
Empirical equations ➔ Problems in Scaling up
NEED OF SIMPLIFCATION: Structure, segregation

Simplified reaction scheme
Many reaction rates, kinetic parameters (macroscopic)
Empirical kinetic model: key components

3. KINETIC MODELS

CÁLCULOS DE LOS PARÁMETROS CINÉTICOS:

- To estimate the values of K_M and v_{max} least squares fitting can be used on Michaelis-Menten linearizations.

-**LINEARIZATION:** transformation of the equation by rearranging its terms, to generate a linear graphical plotting.

SUGGESTED LINEARIZATIONS:

1) Lineweaver-Burk
2) Eadie-Hofstee
3) Hanes-Wolf

3. KINETIC MODELS

TWO SUBSTRATES and TWO PRODUCTS:

A + B + E ⇐⇒ P + Q + E

SITUATION 1: **Formation of one ternary complex**

between substrate A, substrate B and enzyme:

- Random
- Ordered

SITUATION 2: **Ping-Pong Mechanism**

➔ Via binary complexes

➔ First, enzyme gets in contact with one of the substrates so that one first product is generated..

➔ Then, the second substrate enters so that enzyme is released and the second product generated.

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